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Total correction of hemophilia A mice with canine FVIII using an AAV 8 serotype.

Sarkar R, Tetreault R, Gao G, Wang L, Bell P, Chandler R, Wilson JM, Kazazian HH Jr.

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Despite the popularity of adeno-associated virus 2 (AAV2) as a vehicle for gene transfer, its efficacy for liver-directed gene therapy in hemophilia A or B has been suboptimal. Here we evaluated AAV serotypes 2, 5, 7, and 8 in gene therapy of factor VIII (FVIII) deficiency in a hemophilia A mouse model and found that AAV8 was superior to the other 3 serotypes. We expressed canine B domain-deleted FVIII cDNA either in a single vector or in 2 separate AAV vectors containing the heavy- and light-chain cDNAs. We also evaluated AAV8 against AAV2 in intraportal and tail vein injections. AAV8 gave 100% correction of plasma FVIII activity irrespective of the vector type or route of administration.

PMID: 14551134 [PubMed - indexed for MEDLINE]

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Safety and efficacy of factor IX gene transfer to skeletal muscle in murine and canine hemophilia B models by adeno-associated viral vector serotype 1.

Arruda VR, Schuettrumpf J, Herzog RW, Nichols TC, Robinson N, Lotfi Y, Mingozzi F, Xiao W, Couto LB, High KA.

Department of Pediatrics, University of Pennsylvani Medical Center, The Children's Hospital of Philadelphia, Philadelphia, PA 19104, USA.

Adeno-associated viral (AAV) vectors (serotype 2) efficiently transduce skeletal muscle, and have been used as gene delivery vehicles for hemophilia B and for muscular dystrophies in experimental animals and humans. Recent reports suggest that AAV vectors based on serotypes 1, 5, and 7 transduce murine skeletal muscle much more efficiently than AAV-2, with reported increases in expression ranging from 2-fold to 1000-fold. We sought to determine whether this increased efficacy could be observed in species other than mice. In immunodeficient mice we saw 10- to 20-fold higher levels of human factor IX (hF.IX) expression at a range of doses, and in hemophilic dogs we observed approximately 50-fold higher levels of expression. The increase in transgene expression was due partly to higher gene copy number and a larger number of cells transduced at each injection site. In all immunocompetent animals injected with AAV-1, inhibitory antibodies to F.IX developed, but in immunocompetent mice treated with high doses of vector, inhibitory antibodies eventually disappeared. These studies emphasize that the increased efficacy of AAV-1 vectors carries a risk of inhibitor formation, and that further studies will be required to define doses and treatment regimens that result in tolerance rather than immunity to F.IX.

PMID: 12969984 [PubMed - indexed for MEDLINE]

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Sarkar and 2004 and Blood... (1)

1: Hum Gene Ther. 2006 Aug;17(8):807-20.

Enhanced gene transfer efficiency in the murine striatum and an orthotopic glioblastoma tumor model, using AAV-7- and AAV-8-pseudotyped vectors.

Harding TC, Dickinson PJ, Roberts BN, Yendluri S, Gonzalez-Edick M, Lecouteur RA, Jooss KU.

Cell Genesys, South San Francisco, CA 94080, USA. thomash@cellgenesys.com

In this study, recombinant AAV vectors pseudotyped with viral capsids derived from AAV serotypes 7 and 8 were evaluated for gene transfer in the murine striatum relative to vectors pseudotyped with AAV serotypes 2, 5, and 6. In comparison with rAAV serotype 2, pseudotyped vectors derived from AAV-7 and AAV-8 have increased transduction efficiency in the murine CNS, with the rank order rAAV-7 > rAAV-8 > rAAV-5 > rAAV-2 = rAAV-6, with all vectors demonstrating a marked tropism for neuronal transduction. Pseudotyped rAAV vector gene transfer in the brain after preimplantation of a murine 4C8 glioblastoma tumor was also evaluated. Efficiency of gene transfer to the orthotopic tumor was increased when using AAV-6, -7, and -8 capsid proteins in comparison with serotype 2, with the order rAAV-8 = rAAV-7 > rAAV-6 > rAAV-2 > rAAV-5. The increased gene transfer efficiency of rAAV vectors pseudotyped with the rAAV-8 capsid also provided enhanced therapeutic efficacy in a mouse model of glioblastoma multiforme, using vectors encoding an inhibitor of the vascular endothelial growth factor pathway. These studies demonstrate that rAAV vectors pseudotyped with capsids derived from AAV serotypes 7 and 8 provide enhanced gene transfer in the murine CNS and may offer increased therapeutic efficacy in the treatment of neurological disease.

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Adeno-associated virus (AAV)-7 and -8 poorly transduce vascular endothelial cells and are sensitive to proteasomal degradation.

Denby L, Nicklin SA, Baker AH.

British Heart Foundation, Glasgow Cardiovascular Research Centre, Division of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK.

Transduction of the vascular endothelium by adeno-associated virus (AAV) vectors would have broad appeal for gene therapy. However, levels of transduction by AAV serotype-2 are low, an observation linked to deficiencies in endothelial cell binding, sequestration of virions in the extracellular matrix and/or virion degradation by the proteasome. Strategies to improve transduction of endothelial cells include AAV-2 capsid targeting using small peptides isolated by phage display or the use of alternate serotypes. Previously, we have shown that AAV serotypes-3 through -6 transduce endothelial cells with poor efficiency. Recently, AAV serotypes-7 and -8 have been shown to mediate efficient transduction of the skeletal muscle and liver, respectively, although their infectivity profile for vascular cells has not been addressed. Here, we show that AAV-7 and -8 also transduce endothelial cells with poor efficiency and the levels of transgene expression are markedly enhanced by inhibition of the proteasome. In both cases proteasome blockade enhances the nuclear translocation of virions. We further show that this is vascular cell-type selective since transduction of smooth muscle cells is not sensitive to proteasome inhibition. Analysis in intact blood vessels corroborated these findings and suggests that proteasome degradation is a common limiting factor for endothelial cell transduction by AAV vectors.

PMID: 15944729 [PubMed - indexed for MEDLINE]

1: J Virol. 2001 Feb;75(4):1824-33.

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Intracellular trafficking of adeno-associated virus vectors: routing to the late endosomal compartment and proteasome degradation.

Douar AM, Poulard K, Stockholm D, Danos O.

Genethon III-CNRS URA 1923, Evry, France. douar@genethon.fr

The early steps of adeno-associated virus (AAV) infection involve attachment to a variety of cell surface receptors (heparan sulfate, integrins, and fibroblast growth factor receptor 1) followed by clathrin-dependent or independent internalization. Here we have studied the subsequent intracellular trafficking of AAV particles from the endosomal compartment to the nucleus. Human cell lines were transduced with a recombinant AAV (rAAV) carrying a reporter gene (luciferase or green fluorescent protein) in the presence of agents that affect trafficking. The effects of bafilomycin A(1), brefeldin A, and MG-132 were measured. These drugs act at the level of endosome acidification, early-to-late endosome transition, and proteasome activity, respectively. We observed that the transducing virions needed to be routed as far as the late endosomal compartment. This behavior was markedly different from that observed with adenovirus particles. Antiproteasome treatments with MG-132 led to a 50-fold enhancement in transduction efficiency. This effect was accompanied by a 10-fold intracellular accumulation of single-stranded DNA AAV genomes, suggesting that the mechanism of transduction enhancement was different from the one mediated by a helper adenovirus, which facilitates the conversion of the rAAV single-stranded DNA genome into its replicative form. MG-132, a drug currently in clinical use, could be of practical use for potentializing rAAV-mediated delivery of therapeutic genes.

PMID: 11160681 [PubMed - indexed for MEDLINE]

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Proteasome inhibition enhances AAV-mediated transgene expression in human synoviocytes in vitro and in vivo.

Jennings K, Miyamae T, Traister R, Marinov A, Katakura S, Sowders D, Trapnell B, Wilson JM, Gao G, Hirsch R.

William S. Rowe Division of Rheumatology, Children's Hospital Medical Center, Cincinnati, OH 45229, USA.

To explore the potential applicability of recombinant adeno-associated virus (rAAV) vectors in the treatment of rheumatoid arthritis (RA), primary human fibroblast-like synoviocytes (FLS) derived from patients with RA were infected with rAAV encoding mouse IL-10 under the control of the CMV promoter. Addition of the proteasome inhibitor carbobenzoxy-l-leucyl-l-leucyl-l-leucinal (zLLL) to the cultures dramatically enhanced expression of the IL-10 transgene, in a dose-dependent manner. The increased expression was transient, peaking at 3 days and returning to near baseline by 7 days. The enhancement was observed even when zLLL was added 13 days after infection with rAAV. The effect of zLLL was not specific to either the mIL-10 transgene or the CMV promoter, as similar findings were observed using an rAAV construct encoding alpha1-anti-trypsin under the control of the chick beta-actin promoter or GFP, driven by the CMV promoter. Transgene expression could be repeatedly induced by reexposure to zLLL. Transgene mRNA levels increased in parallel with protein levels. Transgene expression could also be repeatedly induced in vivo by administering zLLL to SCID mice previously injected with rAAV-infected FLS. These data demonstrate that proteasome inhibition can dramatically enhance transgene expression in human RA FLS following infection with rAAV and suggest a possible approach to regulating synovial transgene expression in vivo.

PMID: 15771962 [PubMed - indexed for MEDLINE]

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Model system for high-throughput screening of novel human immunodeficiency virus protease inhibitors in Escherichia coli.

Cheng TJ, Brik A, Wong CH, Kan CC.

Keck Graduate Institute of Applied Life Sciences, 535 Watson Drive, Claremont, CA 91711, USA.

Novel human immunodeficiency virus (HIV) protease inhibitors are urgently needed for combating the drug-resistance problem in the fight against AIDS. To facilitate lead discovery of HIV protease inhibitors, we have developed a safe, convenient, and cost-effective Escherichia coli-based assay system. This E. coli-based system involves coexpression of an engineered beta-galactosidase as an HIV protease substrate and the HIV protease precursor comprising the transframe region and the protease domain. Autoprocessing of the HIV protease precursor releases the mature HIV protease. Subsequently, the HIV protease cleaves beta-galactosidase, resulting in a loss of the beta-galactosidase activity, which can be detected in high-throughput screens. Using Food and Drug Administration-approved HIV protease inhibitors, this E. coli-based system is validated as a surrogate screening system for identifying inhibitors that not only possess inhibitory activity against HIV protease but also have solubility and permeability for in vivo activity. The usefulness of the E. coli-based system was demonstrated with the identification of a novel HIV protease inhibitor from a library of compounds that were prepared by an amide-forming reaction with transition-state analog cores. A novel inhibitor with a sulfonamide core of amprenavir, E2, has shown good correlation with the in vitro enzymatic assay and in vivo E. coli-based system. This system can also be used to generate drug resistance profiles that could be used to suggest therapeutic uses of HIV protease inhibitors to treat the drug-resistant HIV strains. This simple yet efficient E. coli system not only represents a screening platform for high-throughput identification of leads targeting the HIV proteases but also can be adapted to all other classes of proteases.

PMID: 15215092 [PubMed - indexed for MEDLINE]

1: J Biol Chem. 2002 Oct 11;277(41):38322-7. Epub 2002 Aug 6.

Identification and biological characterization of heterocyclic inhibitors of the hepatitis C virus RNA-dependent RNA polymerase.

Dhanak D, Duffy KJ, Johnston VK, Lin-Goerke J, Darcy M, Shaw AN, Gu B, Silverman C, Gates AT, Nonnemacher MR, Earnshaw DL, Casper DJ, Kaura A, Baker A, Greenwood C, Gutshall LL, Maley D, DelVecchio A, Macarron R, Hofmann GA, Alnoah Z, Cheng HY, Chan G, Khandekar S, Keenan RM, Sarisky RT.

Department of Medicinal Chemistry, The Musculoskeletal, Microbial and Proliferative Diseases Center of Excellence for Drug Discovery, GlaxoSmithKline Pharmaceuticals, Collegeville, Pennsylvania 19426, USA.

The hepatitis C virus (HCV) NS5B protein encodes an RNA-dependent RNA polymerase (RdRp), the primary catalytic enzyme of the HCV replicase complex. We established a biochemical RNA synthesis assay, using purified recombinant NS5B lacking the C-terminal 21 amino acid residues, to identify potential polymerase inhibitors from a high throughput screen of the GlaxoSmithKline proprietary compound collection. The benzo-1,2,4-thiadiazine compound 1 was found to be a potent, highly specific inhibitor of NS5B. This agent interacts directly with the viral polymerase and inhibits RNA synthesis in a manner noncompetitive with respect to GTP. Furthermore, in the absence of an in vitro-reconstituted HCV replicase assay employing viral and host proteins, the ability of compound 1 to inhibit NS5B-directed viral RNA replication was determined using the Huh7 cell-based HCV replicon system. Compound 1 reduced viral RNA in replicon cells with an IC(50) of approximately 0.5 microm, suggesting that the inhibitor was able to access the perinuclear membrane and inhibit the polymerase activity in the context of a replicase complex. Preliminary structure-activity studies on compound 1 led to the identification of a modified inhibitor, compound 4, showing an improvement in both biochemical and cell-based potency. Lastly, data are presented suggesting that these compounds interfere with the formation of negative and positive strand progeny RNA by a similar mode of action. Investigations are ongoing to assess the potential utility of such agents in the treatment of chronic HCV disease.

PMID: 12167642 [PubMed - indexed for MEDLINE]